STUDIES ON POSSIBLE PROPAGATION OF MICROBIAL CONTAMINATION IN PLANETARY CLOUDS

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One of the key parameters in estimation of the probability of contamination (Pc) of the outer planets (Jupiter, Saturn, Uranus, etc.) is the probability of growth (Pg) of terrestrial microorganisms on or near these planets. For example, Jupiter appears to have an atmosphere in which some microbial species could metabolize and propagate. This study includes investigation of the likelihood of metabolism and propagation of microbes suspended in dynamic atmospheres. It is directed toward providing experimental information needed to aid in rational estimation of Pg for these outer planets. Current work is directed at demonstration of aerial metabolism under near optimal conditions and tests of propagation in simulated Jovian atmospheres.

APPROACH

Theory

Literature on the planetary composition and the atmospheric structure and dynamics of the planet Jupiter includes reports of some firm data, discussions of some theoretically valid concepts, and some speculative ideas. In brief, the most probable structure is that the planet has an atmosphere at least 1000 Km in depth (3), beyond which components may become liquid, or molecular structure may shift to the metallic state (5,15,26). A solid planetary surface, as on earth, is not very probable (5). Solids most certainly exist in the form of particles or crystals, and it is possible that very large masses of these can be formed by agglomeration to "float" in the atmosphere. The "red spot" may be such an agglomerate (8). Jupiter has a magnetic field, and a turbulent atmosphere with electromagnetic and thermodynamic storms (3). All thermal models indicate there is an atmospheric stratum about 80 Km thick where temperatures vary from 0° C to 100° C at pressures from about 2 to 50 earth atmospheres (3,15,26).

The planet consists of 97 - 99% hydrogen and helium in nearly equal masses. The remainder of the planet has been shown to contain mostly methane, ammonia and water with traces of neon (3,5,7).

The fact that there are patches of red, brown and white color in the Jovian atmosphere led to speculation about the presence of other

elements. Unless the planet is very unusual, most other elements should exist there in some quantity, and the effective mass of minor elements could be large (8,9,10). Note that the average abundance of carbon (300 parts per million) on earth, classes it as almost a trace element. Since the Jovian atmosphere is highly reducing, elements such as phosphorus or sulfur would exist as hydrides with appreciable vapor pressures (19).

Clouds of NH_3, NH_3:H_2O, and probably NH_3:H_2S exist just above the biological stratum (8). Rain undoubtedly occurs. The water, if pure, is probably saturated with ammonia (5,7). However, Ponnaperuma and others have shown that a variety of organic compounds are formed by electrical discharges in methane-ammonia atmospheres. Many are soluble in water and could act as buffers to reduce the pH of NH_3:H_2O solutions. The quantity and extent of the organic substances vary with the electrical discharge intensity and gaseous composition (11,16,17,18,19,24,27).

As droplets fall through the biological stratum, the water will evaporate, leaving small, solid particles. Many will be carried aloft to act as condensation nuclei; others will fall into the lower areas where temperatures as high as 1000° C are postulated to exist. At these temperatures, pyrolysis occurs, and CH_4, H_2O and NH_3 would be reformed. Prior to reaching pyrolytic temperatures, however, a variety of other degradation compounds could be formed; some materials would escape as vapor. Thus, the biological stratum is probably rich in organic substances both particulate and gaseous.

If the biological stratum were considered to be a stirred settling chamber, then the half-life (2) of a 2 μm particle of density 1.1 would be about 5 years. That is:

$$\frac{Nt}{No} = e^{-kt}$$

where N = number at time t

No = number at time o

t = time, and

$k = \frac{V}{H}$

where V = Stokes terminal velocity

H = effective height of a volume of air and $t_{1/2} = 0.693 \frac{H}{V}$, where $t_{1/2}$ = half life in seconds.

Substituting the Jovian gravitational constant in Stokes formula and assuming no significant changes in viscosity (η),

$$V = \frac{P\, d^2\, g}{18} \quad \text{or} \quad = \frac{1.1(2\times10^{-4})^2\ 2500}{18\ (180 \times 10^{-6})}$$

$$= 3.4 \times 10^{-2} \text{ cm/sec}$$

$$\text{then} \quad \frac{H}{V} = \frac{8 \times 10^6 \text{ cm}}{3.4 \times 10^{-2}} = 23.5 \times 10^7 \text{ sec.}$$

$$t_{1/2} = 0.69 \times 2.3 \times 10^8 = 1.6 \times 10^8 \text{ sec} = 5 \text{ years.}$$

Under these cirucmstances, any anaerobic microbial species that could properly collect and utilize the organic material to reproduce new individuals at least once every 2.5 years would maintain a stable population (21). Those species with shorter division times would increase in numbers.

Such anaerobic microbes would have to evolve a metabolic and mechanical system that permits wastes and electron donor molecules to be discarded while simultaneously storing elements such as S, P, Fe, Mg, Ca, Na, Cl and water molecules with great tenacity (9,12,13).

There are two major aspects of studies that might determine whether airborne growth in such atmospheres is possible.

1. One can attempt to demonstrate first metabolism and then reproduction in the airborne state under any atmospheric situation.

2. One can attempt to isolate and cultivate an earth-type microbe capable of utilizing electrically-formed compounds in a closed system simulating Jovian conditions. This research has been investigating both aspects.

Experimental

The aerosol apparatus (Fig. 1): Aerosols are contained in a 540 liter drum (6) rotating at 4 RPM held in a temperature-controlled room (±1 C). The drum and seals are air-tight and the non-rotating port contains ports for air inlet and outlet. The inlet side has provisions for mixing wet and dry air to allow desired humidity conditions to be established, as well as dual ports for insertion of aerosols.

Samples are obtained either by a standard AGI impinger, 12.5 liters per min. (for viability studies) or by a bubbler system, 100 ml per min., shown in Fig. 2 (for sampling radioactivity).

Aerosols are produced by a standard, twin fluid, peripheral refluxing atomizer known to produce particles of mass median diameters of near 2μm (2). In one instance, the jet was modified to permit two liquids to mix at a point near the air-jet exit.

The Jovian chamber (fig. 3): The chamber consists of a glass vessel having a central, spherical volume of 3 liters, a vertical tube extending upward about 45 cm and a vertical quartz tube extending downward about 10 cm. The top tube contains a removable "cold finger" through which cold water can be circulated, and a set of electrodes, one (at ground potential) in contact with the finger.

The quartz tube is fitted into a fire-clay and asbestos cylinder containing a nichrome heating element in contact with the tube. This arrangement allows the bottom of the tube to be heated to a bright, cherry-red color.

The cold finger terminates in a thin rod 30 cm long and ending in a hook. A glass filter paper strip 1.5 cm wide and 8 cm long, with a pointed bottom, is affixed to the hook via a hole in the top of the strip.

The unit also contains a port allowing gas exchange and pressure monitoring, as well as a hole 2 mm in diameter covered with an RTV rubber seal through which a long needle can be inserted for sampling purposes. The entire unit is enclosed in a metal mesh container for protection against explosion, as well as to serve as a Faraday shield. A simple Tesla coil with an output of about 30 KV and operated 10 sec during each minute, provides an electrical discharge directed onto the grounded cold finger. Thus, the organic compounds are formed at or near the surface of water collected on the cold finger, and the compounds are made available to microbes which can be planted on the filter strip.

METHODS AND RESULTS

Aerosol Studies

The first approach to determine whether airborne microbes can carry out metabolic functions was to add uniformly ^{14}C labeled glucose to a washed suspension of Serratia marcescens cells just prior to aerosolizing them into the chamber conditioned to a relative humidity of 80% at 22 C. If metabolism occurred, then the cell content of labeled glucose should increase, then decrease, as a function of time, and $^{14}CO_2$ should be formed. This work is being done in collaboration with Biospherics, Inc.

The first experiment was done with a dual-fluid atomizer. The failed because of the lack of effectiveness of that atomizing technique to produce an adequate ^{14}C level per liter of air; 95% of the glucose solution was lost because material not atomized contained a high cell content and could not be re-used.

The second set of experiments was conducted with two atomizers, one containing bacteria and the other tagged glucose. The experiment depended on collision of bacteria with a tagged glucose particle, uptake of glucose and subsequent evolution of $^{14}CO_2$ as a result of glycolysis. The two aerosols were mixed in a 2.5 ft, 1/2 inch diameter tube prior to injection into the chamber. A preliminary experiment wherein aerosols of cells and hypochlorite were mixed, indicated the particulate collision efficiency would be adequate.

The protocol was to establish an aerosol (0 time) and sample at 5 min., 2 hr., 4 hr., and 24 hrs. At each sample time, 3 specimens were taken in rapid succession; an impinger (1 min. duration) and two bubblers (1 min. each). The filter disk preceding the bubbler was washed, glucose was added, and the disk incubated in a closed chamber with a CO_2 adsorbent to determine whether collected cells produced $^{14}CO_2$.

The second disk was washed, digested and counted to determine the amount of glucose incorporated into cells. Assay of radioactivity was done with standard scintillation counting fluids and techniques.

Results of the first test were essentially inconclusive. Viability assays indicated the overall loss of living, airborne cells to be 1/2 log in 24 hrs. and this value includes loss of particles to walls. The total radioactivity collected per sample, a measure of airborne particulate matter, showed a decrease of about 1/3 log in 24 hrs. Thus, a slight loss of numbers of viable cells (biological decay) had occurred at 90% relative humidity.

As samples were assayed, it became evident that the filters, or fliter holders, were leaking. A run was made with glucose alone to determine the percentage of particles lost through the filter. When ratios of the soluble fraction to that retained on the filter are compared statistically, there was a significant difference between samples in the first run and the glucose-only set ($t = 2.59$), but not in the second run. In a third run, after filter leakage had been eliminated by employing 0.1μm type membrane fliters, and using 10μc/ml of glucose, we found significant $^{14}CO_2$ evolution had occurred during the first 10 minutes; none occurred thereafter. This is presumptive evidence of collision, glucose uptake, and metabolism in the airborne state.

Jupiter Environment

The first task was to isolate ammonia-tolerant anaerobes (4,22, 26). Guano samples, as well as Guano beetles, were obtained from the Frio bat caves in Texas, where often ammonia vapor is sufficiently high to be toxic to the human (20,25). Samples of various local soils were also obtained.

Enrichment cultures were made by mixing the samples with distilled water and placing the mixtures in anaerobic jars with a small beaker holding 10 ml of 30% solution of ammonium hydroxide. The jar was evacuated 5 times to 1/2 atm, sequentially replacing each lost volume with nitrogen. Samples were removed after 5-10 days, plated on nutrient agar and plates were incubated as above.

Another method was to provide deep-poured tubes of nutrient agar, to overlay the agar with 1 ml of the ammonia solution, and to stab the sample through the solution and deep into the agar with an inoculating needle.

Almost all samples yielded mixed populations of bacteria that would grow anaerobically on nutrient agar in the presence of ammonia. Surprisingly, the culture that grew most abundantly within two days was found in soil sample obtained from a small garden near the entrance to the laboratory.

Cultures obtained in this way were sequentially transferred every 3 days for a period of 4 months. Colonies were deliberately intermixed for the purpose of stimulating synergistic growth.

A sample of the mixed population was inoculated onto a moistened filter strip, the strip was hung on the glass hook described above and placed in the Jovian chamber. The chamber was flushed 5 times with nitrogen and supplied with about 2% by volume of ammonia and methane gas plus about 50 ml of H_2S and 2 ml of H_2O. No hydrogen or helium was used in these initial experiments. The pressure was maintained at one atm.

Water at 5 C was circulated through the cold finger and the electric discharge was started. In about 30 minutes, sufficient moisture had been collected on the finger, and had run down the rod to the strip, to cause the strip to be saturated and liquid drops started falling into the heated quartz tube. After that time, moisture continuously ciruclated and the air mass was being mixed as a result of sudden rush of steam from the falling drops.

Several experiments were conducted in this manner. In the first experiment, the strip was removed after 24 hrs., placed on nutrient

agar, and incubated in an anaerobic jar. Growth occurred after 4 days, indicating some cells had survived and had been held by the glass fibers. Organic matter was being formed because dark-brown material collected on the top of the quartz tube, and a red-brown coloration appeared in water droplets condensing on the side of the vessel.

In another experiment the strip was removed after 4 days, but no growth occurred after 4 additional days of anaerobic incubation on nutrient agar. The plate with the strip was then removed and allowed to incubate at room temperature on the bench. Abundant aerobic growth occurred after 3 days.

This indicates that cells were held by the strip for at least 4 days and that the glass paper strip is a suitable holding matrix. It is evident that the environment, probably the electric discharge products, inhibited any obligate anaerobic forms that might have been in the population and, at the same time, inhibited the anaerobic metabolism of any facultative forms.

DISCUSSION

Metabolism, per se, is not necessarily proof that the cell can propagate. Yet, without metabolism propagation is impossible. It is probably easier to demonstrate metabolism than division, for living processes may be very slow in airborne cells, and there is a limit to how long particulate matter can be held airborne on earth. Hence, metabolism should be the first demonstrable activity. If it can be conclusively proven, the metabolic rate should help to determine how long a potential division process might require.

There are ways other than use of radioactive tracers for indicating whether metabolism has occurred. A change in the DNA protein ratio might be one; loss or gain of specific enzyme activity might be another. Deficient mutants capable of induced enzyme formation or of genetic cross-linkage might also be utilized.

The simulated atmosphere of Jupiter was not immediately lethal to common vegatative cells. Presumably, spores would be no less sensitive. However, survival alone is not the important question, since survival is inherently limited. An important question is whether cells can propagate in atmospheres utilizing traces of organic material formed by electrical discharges. [Note that methane cannot be used as an energy source without oxygen (4)]. The reason the question is important is that if microbes can propagate under these conditions, then if a single cell (or more) were released, the most reasonable Pc value is 1. Of course, many other factors are involved. Growth on a moist

surface is certainly not equivalent to growth in a gas. The cell must be able to obtain and hold moisture, withstand high pH or regulate the pH of its envelope, create and dispose of a suitable energy sink, conserve essential elements and control waste products, to mention a few.

FUTURE WORK

The tracer experiments will be continued, but at increased humidity and at temperatures providing optimal conditions for metabolism. At the same time, we will initiate parallel aerosol experiments using strains of bacteria containing inducable enzymes and with other mutant strains which produce identifiable daughter cells. This work will be done at conditions optimal for the microbial systems to be employed (variants of E. coli) as models.

The effect of the rotating drum configuration and speed of particle fallout will be examined to determine whether it is possible to increase efficiency. Present drum design allows aerosols of 1 μm particles to be suspended in useful quantities for nearly 2 weeks. It is possible that this could be doubled and aerosols could be studied for nearly a month; time enough, perhaps, to allow slow propagation mechanisms to become evident.

We will obtain cultures of spores found on and around the launch facilities and select anaerobic ones, if any (1,14). These will be tested for growth in the products formed in the Jupiter chamber. The influence of the electrical discharge duration, and of the gas composition on the ability of formed products to support growth will be examined.

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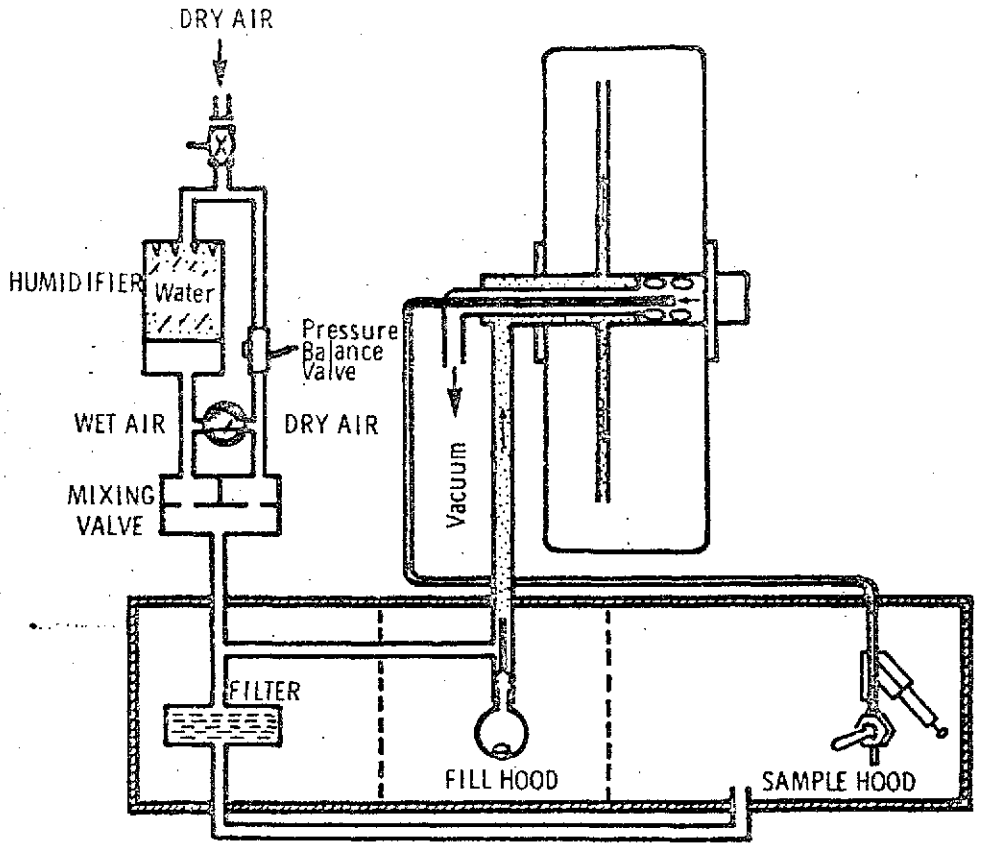

Fig. 1. ROTATING DRUM SYSTEM

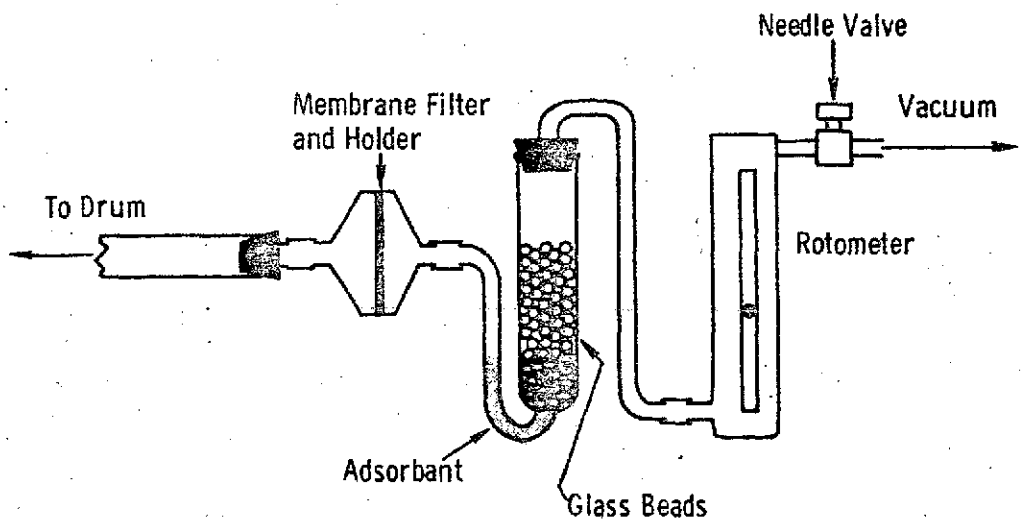

Fig. 2. BUBBLER SAMPLER FOR COLLECTION OF CO_2

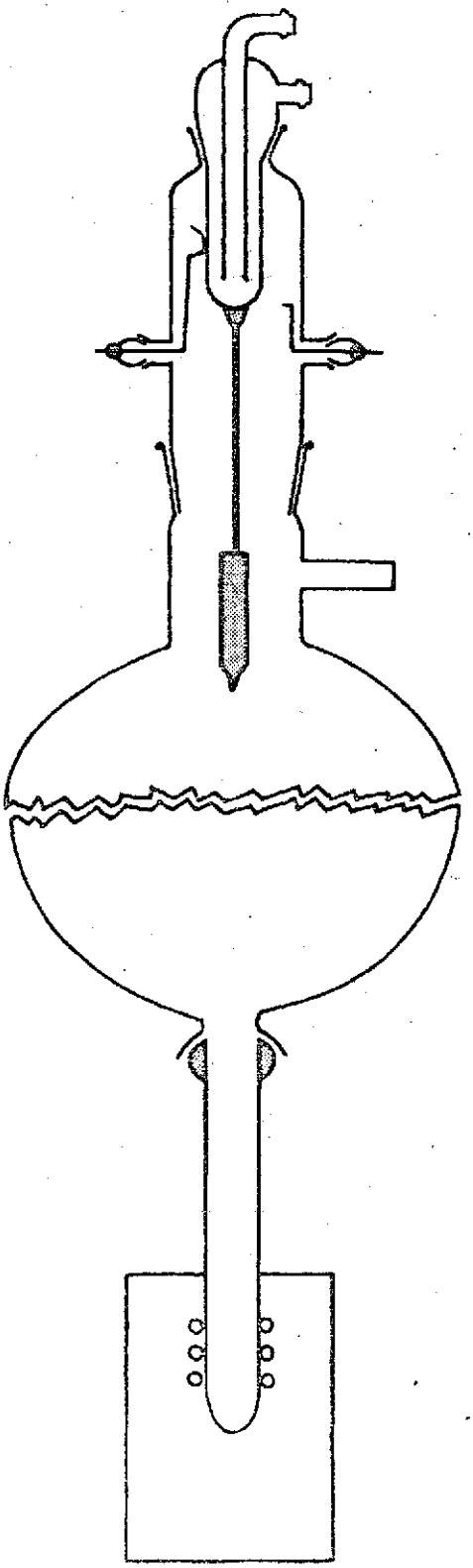

Fig. 3. JUPITER CHAMBER